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Biomedical Engineering

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Transformation into E. Coli XL10-GOLD

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1 Transformation into E. Coli XL10-GOLD

Estimated bench time: 40 minutes
Estimated total time: 2.5 hours
Purpose: Placing the plasmid into bacteria for amplification.

1.1 Materials

- Beta-mercaptoethanol
- Brunsen burner
- Bucket with ice
- E. Coli XL10-GOLD bacterial cells
- Falcon culture tube
- Heat/shaking block
- Mini centrifuge
- Plasmid
- Pipettes and tips
- SOC medium
- Water bath

1.2 Setup & Protocol

- Switch on the water bath and set temperature at 42 °C. Also turn on the heat/shaking-block and set up to 37 °C.
- Load a bucket with ice from the ice machine. Place for each transformation a 14 mL Falcon culture tube on the ice.
- Take the bacterial cells out of the -80 °C freezer. Transfer the cells directly to ice. Do not touch the bottom of the tube that contains the cells.
- Thaw the cells on ice for ~5 minutes.
- Transfer 45 µL of cells to each Falcon culture tube. Keep the tube with cells on ice.
- Add 2 uL of the β-ME mix provided with the kit to the 45 uL of cells. Mix the contents of the tube gently by pipetting up and down. Incubate the cells on ice for 10 min, mixing gently every 2 min.
- Transfer 1.5 µL of PCR mixture to the 45 µL bacterial cells. Mix well. Make sure you work near the Bunsen burner flame.
- Leave the cells on ice for 30 minutes.
- Preheat SOC medium to 42 °C.
- Heat shock the cells for 30 seconds (exactly!) at 42 °C.
- Return the cells directly to ice and incubate for 2 minutes.
- Add 500 µL of SOC medium to the bacteria.
- Incubate for 60 minutes at 37 °C and 250 rpm.